Original Article

Antibacterial Activity and Phytochemical Analysis of *Moringa oleifera* Extract against *Staphylococcus aureus* Identified by Routine and Molecular Methods

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Abstract

Background: Many diseases caused by bacterial and viral infections may now be treated using herbal extracts instead of chemical drugs. **Objectives:** This study aimed to investigate the antibacterial effect of *Moringa oleifera* leaf extracts against the bacterium employed in the experiment, as a commensal. *Staphylococcus aureus* can be found on the skin and in the nasal flora. It can also cause invasive, localized illnesses. Many virulence factors are present in *S. aureus*. It is recognized by routine and molecular methods depending on *Vick* Staphylococcal gene. **Material and Methods:** Phytochemical analysis of high polarity solvent leaves was done by using ethanol. Phytochemical study revealed the presence of tannins, alkaloids, flavonoids, steroids, saponins, and other compounds in the extract. Using the well-diffusion method, the antibacterial effect of the extracts on microorganisms was investigated. **Results:** The ethanol extract proved potent against pathogenic microorganisms, with *S. aureus* showing the highest activity (10–100 mg/mL). In comparison to the concentrations of alcoholic extracts, the zones' inhibition of bacterial growth in diameters increased. However, dosages of 80–100 mg/mL were highly effective and significant against *S. aureus* growth, whereas concentrations of 10–20 mg/mL had low post-detected efficiency and concentrations of 40–60 mg/mL had medium post-detected efficiency. **Conclusion:** These studies demonstrate the validity of the plant's traditional medicinal properties.

Keywords: Antibacterial properties, *Moringa oleifera*, phytochemical screening, *S. aureus*

Introduction

The creation of new antibiotic medicines has become increasingly important due to rising bacterial resistance and a corresponding decline in antibiotic discovery. Combinational strategies may be successful in overcoming resistance and enhancing the effectiveness of conventional antibiotics that are ineffective against resistant bacteria on their own.

Moringa oleifera is a family of Moringaceae plant that is native to South Asia, Africa, the Himalayan Mountains, Pakistan, India, and the Caribbean and Pacific Islands. *M. oleifera*, identified as horseradish trees and drumsticks, ben oil trees, miracle blossom, and "mothers" best companions,' has been naturally identified in various tropical and subtropical locations throughout world.[1] *M. oleifera* is commonly referred to as "drumstick." In the sub-Himalayas, it is a small to medium-sized tree that grows to be approximately 10 m tall.[2] *M. oleifera* is a tiny, fast-growing evergreen or feeding tree with open corn and delicate roots, feathery foliage and inborn leaf, and thick corky, white bark that grows up to (10–12 feet long).[3] It has unique combination of quercetin, zeatin, kaempferol, and other phytochemical flavonoids.[4] It is also a rich source of vitamin B and one of the greatest sources with mineral.[5] *M. oleifera* leaf with ethanol extracts containing niazirin

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and niazirinin, A, B.[6] The methanolic alcohol extract of *Moringa oleifera* leaves contained benzoic, beta benzaldehyde and gallic acid.[7] *M. oleifera* leaves have been shown to contain a number of organic substances that exhibit hypocholesterolémic, antidiabetic, and blood pressure-lowering effects.[8] The *M. oleifera* leaves have a thyroid hormone.[9,10] *M. oleifera* is reported to have anti-inflammatory agents,[11] anti-tumor activity,[12] hepatoprotective effect, anti-inflammatory, antimicrobial, diuretical,[13] antibiotic,[14] hypotensive,[15] antitubercular effect, includes isoniazid or rifampicin,[16] and analgesic effects.[17] It has antispasmodic, in the past for sedative, sputum, and diuretic uses.[18] Some authors reported the effect of *M. oleifera* as anthelmintic ethane extract obtained in adult Indian earthworms at different doses.[19] *M. oleifera* is a plant that has been used in herbal traditions to treat asthma.

In this study, the effectiveness of *M. oleifera* extract against *S. aureus* which is identified by routine and molecular methods depending on specific gene was described.

Material and Methods

The leaf extract preparation was purchased in the Iraqi market. It was assured that the factory will be hygienic and clean. After being washed with tap water, the leaves were properly cleaned and dried to remove dirt and other foreign materials. 30 grams of fresh leaves are boiled for an hour in 200 mL of solvent to create leaf extracts. The extract was vacuumed with a revolving vaporizer at 40–50°C after being filtered with Whatman filter paper No. 1. The solvent was evaporated in the rotary evaporator. It requires the removal of a solvent from crude extract to get the soluble components, and then stored in a refrigerator until use for phytochemical and antibacterial testing.

Bacterial test organism

Gram-positive bacteria (*S. aureus*) were among the test organisms employed in this study. The bacterium was identified and studied in Iraq's Al-Ramadi Hospitals.

DNA preparation

Method of Bacterial DNA extraction[20]:
Isolation of DNA was achieved using the Genomic DNA mini kit Provided by the Wizard® Genomic DNA purification kit (Promega, Wisconsin, United States).

Preparation of leaf extracts

Twenty to thirty grams of fresh *M. oleifera* leaves were weighed and shade-dried for five days at room temperature (32–35°C). The dried leaves were ground into an exceptionally fine powder using a mortar and pestle. Using the Soxhlet method, 25 g of powdered leaf is put in conical flasks and then 500 mL of 90% ethanolic alcohol was added after end extraction. A rotary evaporator was used for removing solvent to concentrate the extract. The extracts were filtered on different sterile Whatman no. 1 filter papers. These extracts are subsequently put through further processing.

Phytochemical analysis

- The presence of flavonoids, alkaloids, volatile oil and steroids, "glycoside, sugar reduction," saponins, and tannins in *M. oleifera* leaf extract was investigated using phytochemical component analysis. In accordance with the procedure established.[21]
- Hager and Baljet test form research was used to show alkaloid participation.
- 3 mL of each extract was added to 10 mL of distilled water and the extract. 1 mL from 10% NaOH was added to solution while the solvent was being shaken. Combination. The appearance of yellow was thus seen as a sign that flavonoids were involved.
- In a test tube, 3 mL from extract was added, diluted with 2 mL of distilled water, and the solution was rapidly shaken. Saponin was identified when a large quantity of bubbles formed.[21]
- Five drops of concentrated H_2SO_4 was added into 1ml of each extract for testing in a specific tube. As a good response, the production of a reddish-brown color was used.
- In a separate test tube, 2 mL from extract was gently boiled for 2 min before cooling. Every single extract received three drops of ferric chloride solution.
- One sample had a 5 mL test tube extract heated to 15 min, cooled for 10%, and neutralized with NaOH before 5 mL Fehling solutions were added. Another sample had a 25 mL diluted sulfuric acid addition, followed by 5 mL Fehling solutions.

Determination of antibacterial activity of *M. oleifera*

- After being re-cultivated on nutritional agar and kept at 37°C for 24 h in the incubator, the bacteria are employed in sterilized tubes filled with heart infusion broth and kept there for 24–72 h. When the light transmittance for the nutrient broth used to assemble the microorganism was 100%, the odds of mild transmittance using the spectrophotometer were only 27% at a wave length of 580 nm.[22]
- The preparation dilution was done by using ethylene glycol, which is an inert microorganism solvent, and by using serial amounts of 10–100 mg of the concentrate extract, then reducing it with ethylene glycol and finishing the quantity to 2 mL to get 1–10% of the final concentrations.
- Antibacterial activity screening was conducted by a good diffusion technique.[17] The plates of the Mueller-Hinton agar were seeded with 0.1 ml of uniform

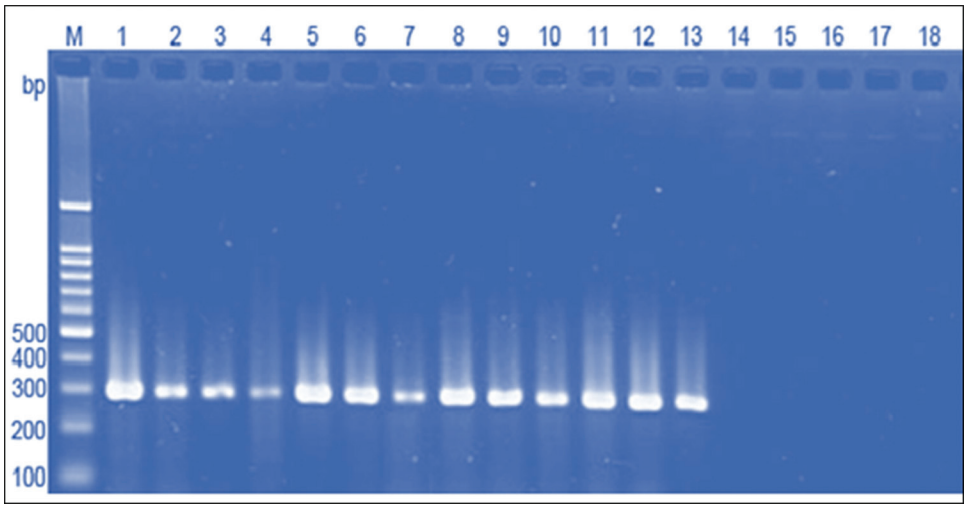

Figure 1: Analyzing the PCR results made with *S. aureus vicK* gene primers using electrophoresis. Amplified DNA products from *S. aureus* were found in lanes (1–13)

bacterial inoculum. The inoculums were flippantly unfolded with a sterile glass spreader over the dish. Within the incubator, the seeded plates were permitted to dry for 20 min at 37°C. In the well with ethylene glycol as manipulate, a chosen crack edge of 9 mm dimension was added to cut widespread wells on the surface of plates, and 0.1 mL of any attention modified into given. For 24 h, the inoculated discs were incubated at 37°C and the area of inhibited diameters was changed to the nearest millimeter.

Results

Identification of bacterial isolates

Staphylococcus aureus isolates had been identified morphologically and by biochemical tests and confirmed by molecular methods using PCR. The morphological identification included microscopic examination by gram stain, cultivation on blood agar and mannitol salt agar, as well as by biochemical tests such as catalase, gelatinase, coagulase (+ve), and oxidase (–ve).

Oligonucleotide primers and PCR procedure

The signal transduction gene vicK, a diagnostic marker for the S. aureus species, served as the foundation for the creation of oligonucleotide primers. The primers' 5′-CTAATACTGAAAGTGAAACGTA-3′ and 5′-TCCTGCACAATCGTACTAAA-3′ sequences made it easier to amp up a 289-bp DNA fragment from exclusively *S. aureus*.

A 50 µL reaction mixture containing the 10-ng genomic DNA template, 1.0 U Taq DNA polymerase (Tiangen Biotechnology Corporation, Beijing, China), 5 µL of 10 PCR buffer, 50 µM dNTPs, 25 pM each primer, and double-distilled water to the final volume of 50 L was used for each DNA amplification in 200 µL microtubes. As a negative control, the reaction mixture without any template DNA was employed. All of the amplifications were performed in a PCR system PTC-200 with an initial denaturation at 94°C for 5 min. This was followed by 35 cycles of denaturation at 94°C for 40 s, primer annealing at 50°C for 40 s, and extension at 72°C for 1 min. After completion of all cycles, 6 µL of 10× DNA loading buffer was added to each tube, and the amplified products were examined in 1.5% agarose gel electrophoresis in the presence of ethidium bromide (0.5 µg/mL). Under UV illumination, the stained gels were seen, and a Las300 Fuji Film was used to take pictures.

Specificity

When DNA from other organisms (*S. epidermidis*, *S. haemolyticus*, *Enterococcus faecalis*, *Enterococcus faecium*, and *E. coli*) were utilized as a template for the PCR reaction, the PCR test specifically amplified a 289 base pair product from S. aureus and did not yield amplicons [Figure 1].

The inhibitory effect of *M. oleifera* extract

The previously identified microorganism's sensitivity steadily grew to the amount of extract from *M. oleifera*. The zone of inhibition for 10 mg/mL was 8 mm, whereas the zone for 100 mg/mL was 23 mm. Despite the fact that the 80–100 mg/mL concentrations were extremely great when compared to the regulation of ethylene glycol as a control, the 10–20 mg/mL concentrations were a substitute for the little vibrant defense against *S. aureus*, whereas the 40–60 mg/mL concentrations were average involvement [Table 2; Figure 2]. The widths of the pathogenic bacterial inhibition zone and the concentrations of *M. oleifera* extract were found to be proportionately correlated.

Table 1: Phytochemical analysis for ethanolic extract of *M. oleifera*

Solvent used for extraction	"Alkaloid	"Flavonoid	"Saponin	"Steroid	"Tannin	Glycoside"
Ethanol	+	+	+	+	++	++

Table 2. Inhibitory effect of different *M. oleifera* extract concentrations in vitro on *S. aureus* measured by inhibited zone dimension (mm)

Conc. (mg/mL) Zone of inhibition (mm)	100	80	60	40	20	10
M. oleifera extract	23	20	16	14	10	8

Figure 2: The effect *M. oleifera* extract as inhibitory for different concentration in MIC on *S. aureus*

Discussion

Considering a rise in bacterial resistance and a corresponding fall in antibiotic discovery, the development of novel antibiotic medications has assumed growing importance. The *M. oleifera* vine, as indicated in Table 1, shows the phytochemical components such alkaloids, flavonoids, glycosides, sugars, proteins, tannins, saponins, terpenoids, and anthrax quinones in solvent extracts. *M. oleifera* was reported to have antimicrobial effect against *S. aureus* at varied concentrations.[17] In varied concentrations, the ethanol leaf extract showed highest efficacy against *S. aureus*, and this agrees with the result shown in Table 2.[2]

Alkaloids are naturally occurring organic molecules with simple atoms of nitrogen. They have pharmacological effects and are used as both recreational and medicinal medications.[18] Flavonoids increase the action of vitamin C and role as antioxidant. They are also thought to have biological activity against pollutants in the liver, tumors, viruses, and other microorganisms.[19] Plant terpenoids are commonly used for their fragrant qualities. They have a role in traditional medicinal herbs, and its antibacterial, tumors, and other medicinal properties are being investigated.[23] Tannins have been found to have antiviral, antibacterial, and antiparasitic effects, and saponins cause red blood cell hemolysis.[24] "Antibacterial activity" was investigated for its possible treatment value against pathogenic bacteria. In earlier tests, the *M. oleifera* medicinal plant showed high antibacterial action against *S. aureus*.

The opportunity to conduct a thorough analysis of molecular processes, virulence, and pathogenesis at the microbial genome levels is greatly enhanced by the recent description of the complete genome sequences of *S. aureus* strains. Additionally, a key component of modern genome analysis is the systematic comparison of genomic sequences from other organisms. Comparative investigations of *S. aureus* sequences can reveal conserved non-coding areas, such as regulatory elements and species markers, as well as coding and coding regions. The signal transduction gene, which was used in the prior method, is referred to the species-specific diagnostic marker in the identification of *S. aureus*.[25] The plant "*Moringa oleifera*" is the most popularly grown plant in the *Moringaceae* family and an essential medicinal herb is *M. oleifera*. Pharmacologically, the antibacterial effects of extracts on the microorganisms investigated in various human situations differ. *M. oleifera* leaf ethanolic extract had the largest spectrum of action on test microorganisms, and *M. oleifera* extracts showed antibacterial activity on bacterial isolates. *M. oleifera* is a great source of several chemical compounds, including alkaloids, flavonoids, glycosides, saponins, and tannins, as shown in Table 1. This study specifically shown the antibacterial activity of *M. oleifera*

against a number of test species, including *Pseudomonas aeroginosa, E. coli, S. aureus, Proteus vulgaris, St. mutans,* and *Bacillus subtilus*. Some individuals utilize *M. oleifera* leaf to treat and prevent malnutrition in addition to traditional medical conditions.[19]

The compounds included in *M. oleifera* leaves may have both curative and preventative effects. More pharmacological study on extracts is required to determine *M. olifera's* effectiveness against more species.[26] The VITEK 2 is an automated microbiology system used for microbial diagnosis and antibiotic sensitivity. Following primary isolation bacteria, a bacterial suspension was created by transferring of some bacterial colonies into two sterile plastic or glass tubes with 3 ml of normal saline and thoroughly mixing them together until observation the turbidity and measured by the Vitek Densichek apparatus (0.5–0.65) for gram positive and negative bacteria. Next, the suspension and card were connected by a microchannel and entered into the Vitek 2 system, for the first time.[27]

Conclusion

The results of this study concluded the validity of the traditional plant's as *M. oleifera* for treating many disease conditions because it has medicinal properties.

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Nil.

Conflicts of interest

There are no conflict of interest.

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